FP-66828-5-PC

## DECODING 16 PROBES
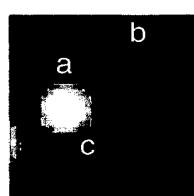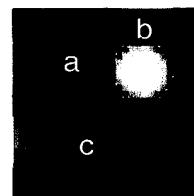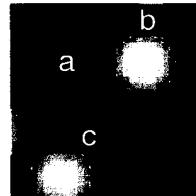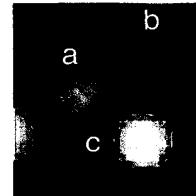
HYB STAGE # 1 2 3 4

|	1	2	3	4
a	GREEN	GREEN	RED	GREEN
b	RED	GREEN	GREEN	RED
c	RED	RED	RED	GREEN

PROBE SEQUENCE 1–16

FIG._1

STAGE 1 STAGE 2

128 BEAD TYPES (4013 BEADS TOTAL)

FOUR DYES (BoD493, BoDR6G, BoD564, BoDTXR)

FOUR STAGES
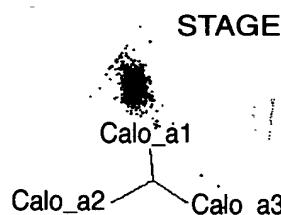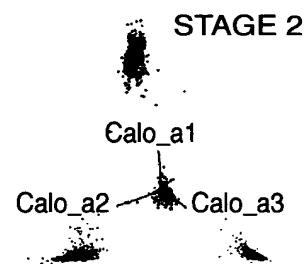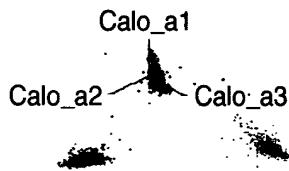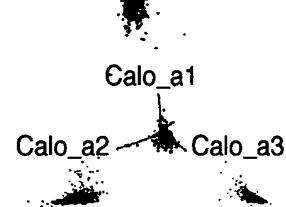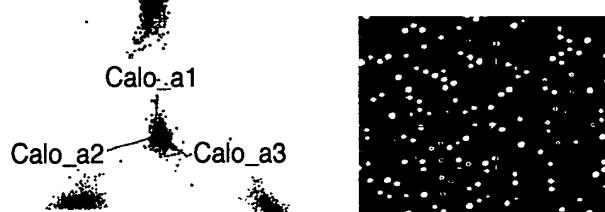
Calo_a1
Calo_a2 Calo_a3

STAGE 3 STAGE 4

Calo_a1
Calo_a2 Calo_a3

FIG._2
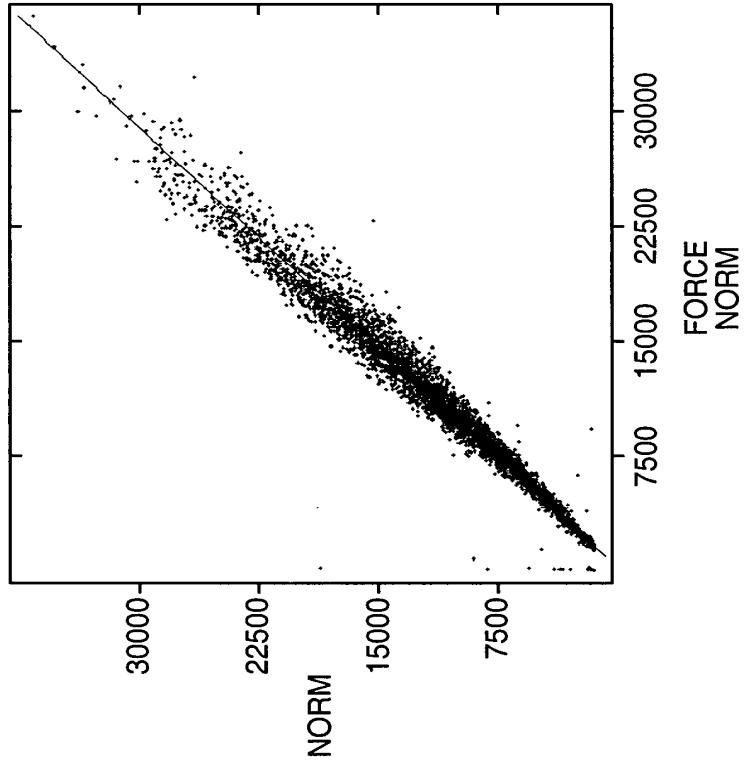
FIG._3B

CODE	S1	S2	S3
1	100	100	100
2	100	100	40
3	100	100	10
4	100	40	100
5	100	40	40
6	100	40	10
7	100	10	100
8	100	10	40
9	100	10	10
10	40	100	100
11	40	100	40
12	40	100	10
13	40	40	100
14	40	40	40
15	40	40	10
16	40	10	100
17	40	10	40
18	40	10	10
19	10	100	100
20	10	100	40
21	10	100	10
22	10	40	100
23	10	40	40
24	10	40	10
25	10	10	100
26	10	10	40
27	10	10	10

FIG._3A

FIG._3C

COUNTS — ALPHA VALUE — S-1

FIG._3D

COUNTS — ALPHA VALUE — S-2

FIG._3E

COUNTS — ALPHA VALUE — S-3

FIG._4

BOD-493

Calo_a1

BOD-TXR

Calo_a2

Calo_a3

BOD-R6G

BOD-564
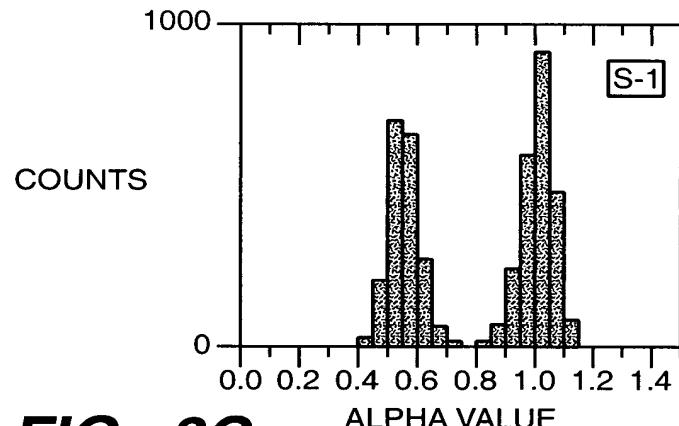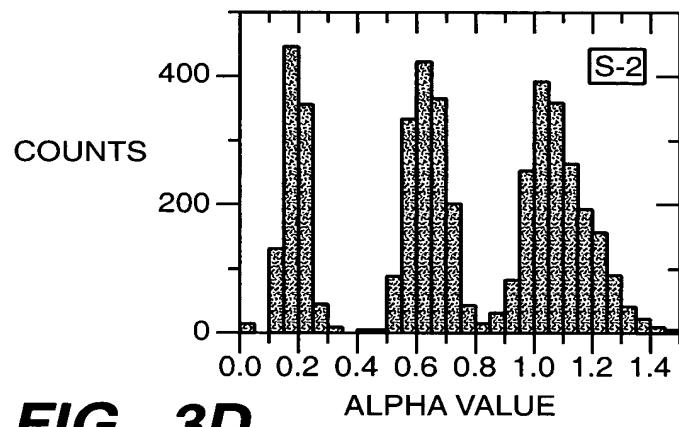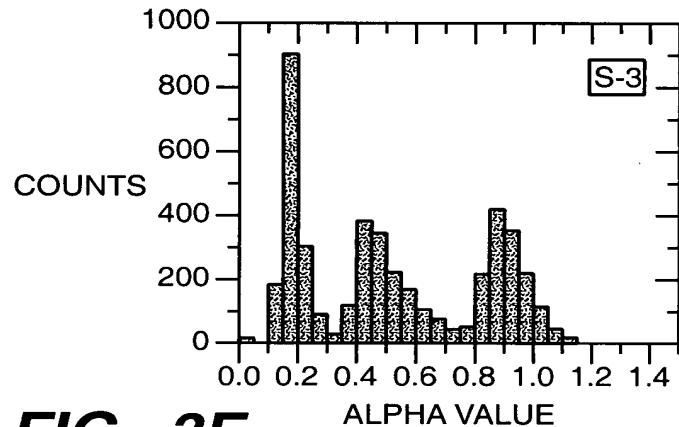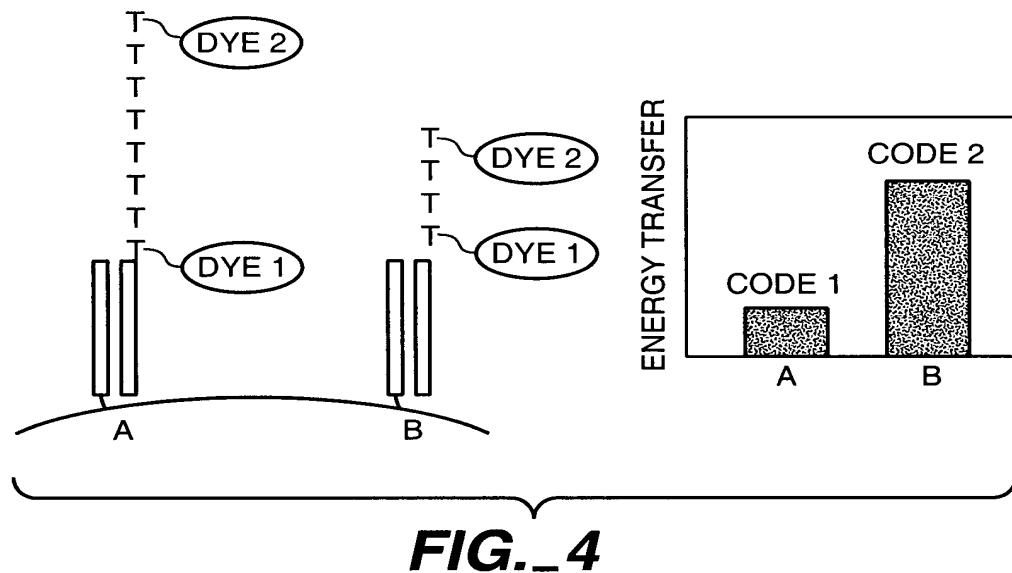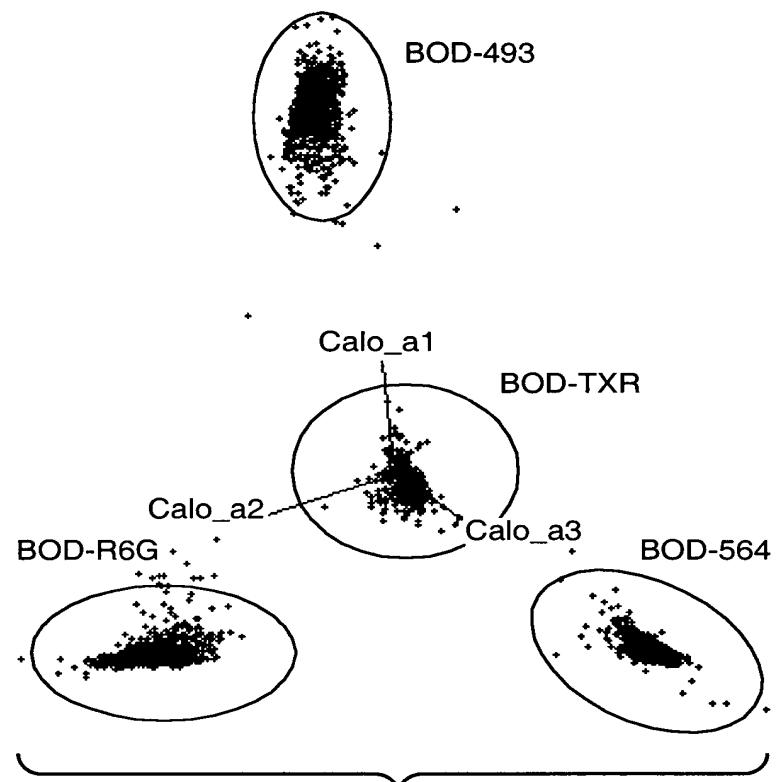
FIG._6

ENERGY TRANSFER SERIES: NORMALIZED TO 1000 AT 530nm LS50B SOLUTION SCREEN

Legend:
- SERIES 1
- SERIES 2
- SERIES 3
- SERIES 4
- SERIES 5
- SERIES 7

Y-axis: NORMALIZED INTENSITY (0, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000)

X-axis: nm (450, 500, 550, 600, 650, 700)
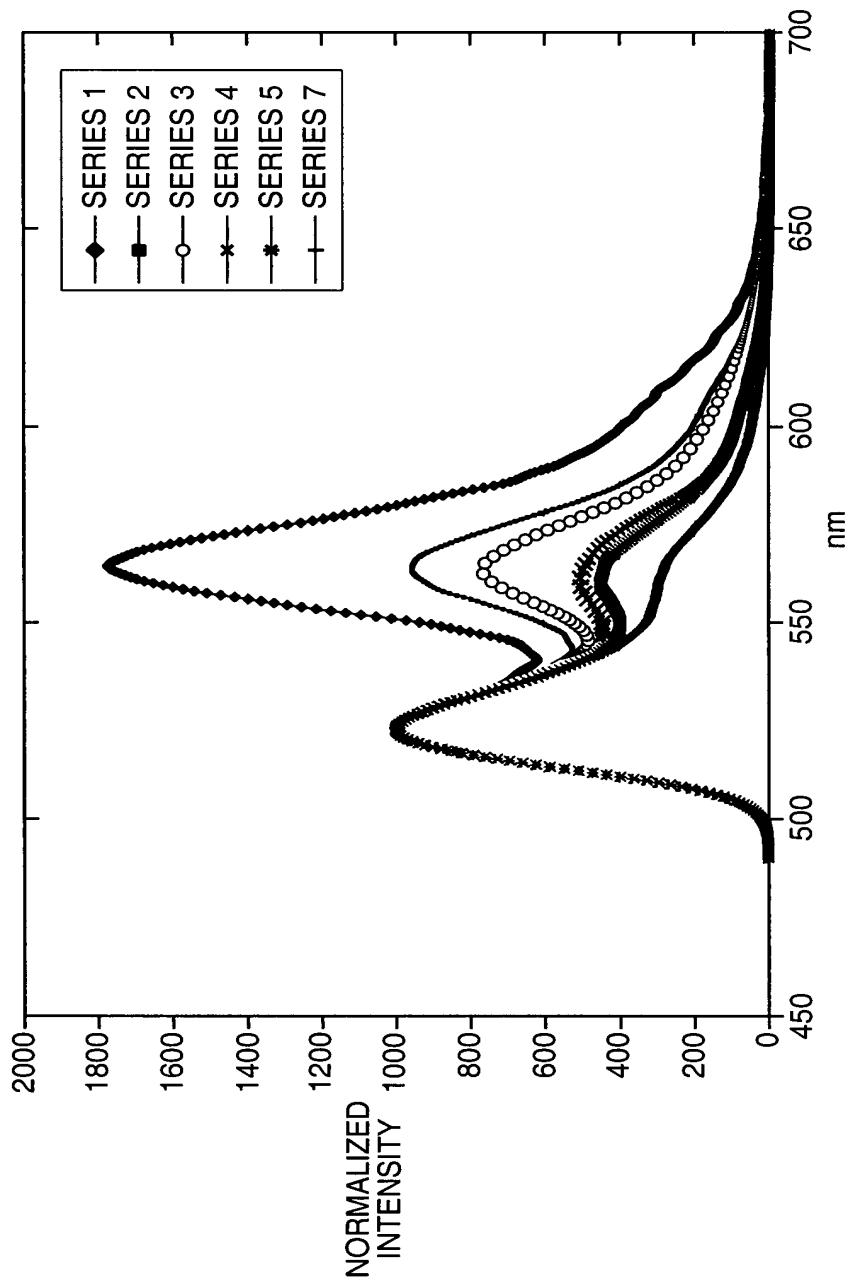
**ENERGY TRANSFER EVALUATION 5' LABELING**

ET1 T**G*CACGAGAATGGAGGTATCT:SERIES 1
ET2 C**TGTCGC*ACGAGAATGGAGGTATCT:SERIES 2
ET3 C**TGTCGGGGCGC*ACGAGAATGGAGGTATCT:SERIES 3
ET4 C**TGTCGGGGCACTCAC*ACGAGAATGGAGGTATCT:SERIES 4
ET5 C**TGTCGGGGCACTCATTGTGC*ACGAGAATGGAGGTATCT:SERIES 5
ET7 C**TGTCGGGGCACTCATTGTCTGTCGGGGCGC*ACGAGAATGGAGGTATCT:SERIES 7

** IS Cy3 AND * IS FLUORESCEIN

FIG._5

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BEAD	1 "COLOR" DECODING				CODE	PARITY
	STAGE 1	STAGE 2	STAGE 3	STAGE 4		
1	1	1	1	1	1111	EVEN
	1	1	1		1110	ODD
	1	1		1	1101	ODD
2	1	1			1100	EVEN
	1		1	1	1011	ODD
3	1		1		1010	EVEN
4	1			1	1001	EVEN
	1				1000	ODD
		1	1	1	0111	ODD
5		1	1		0110	EVEN
6		1		1	0101	EVEN
		1			0100	ODD
7			1	1	0011	EVEN
			1		0010	ODD
				1	0001	ODD
8					0000	EVEN

1001 DIGIT SUM = 2, EVEN PARITY

1011 DIGIT SUM = 3, ODD PARITY

FIG._7

FIG._8

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FIG._9

STAGE 1 EXTENSION

—NH—

$N_1 N_2 N_3$

OH

n = PRIMER SIZE IN nt

STAGE 2 EXTENSION

—NH—

$N_1 N_2 N_3$

OH

n + 1 PRIMER SIZE IN nt

STAGE 3 EXTENSION

—NH—

$N_1 N_2 N_3$

OH

n + 2 PRIMER SIZE IN nt

EACH 'N' WITHIN THE VARIABLE REGION CAN CONTAIN ONE OF 10 POSSIBILITIES:

N = A, G, C, T, A/C, A/G, A/T, C/G, C/T, G/T

FIG._10

FIG._11

CHANNEL 2

CHANNEL 1